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Age determination of Atlantic cod (*Gadus morhua*): Results from an otolith exchange between Canada and France

Détermination de l'âge de la morue (*Gadus morhua*) : Résultats d'un échange d'otolithes entre le Canada et la France

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ABSTRACT

Atlantic cod (*Gadus morhua*) in NAFO subDivision 3Ps are exploited and jointly managed by Canada and France. Assessments of stock status incorporate age determinations from both countries when dis-aggregating total catches into catch-at-age. An otolith exchange was initiated to evaluate the degree of consistency in the age determinations of Canadian and French personnel. Both graphical and statistical analysis clearly indicate differences in interpretation between Canadian and French readers, with ages assigned by Canadian personnel generally being one-year greater than those of participants from France. However, consistency in age determinations was relatively high within Canada and within France.

RÉSUMÉ

Les morues (*Gadus morhua*) dans la sous-division 3Ps de l'Organisation des pêches de l'Atlantique Nord-Ouest (OPANO) sont exploitées et gérées conjointement par le Canada et la France. Les évaluations de l'état des stocks comprennent la détermination de l'âge réalisée par les deux pays en subdivisant les captures totales en captures à l'âge. Un échange d'otolithes a été effectué afin d'évaluer le niveau de cohérence dans la détermination de l'âge par le personnel canadien et le personnel français. Tant l'analyse graphique que l'analyse statistique indiquent clairement des différences d'interprétation entre les lecteurs canadiens et français; le personnel canadien détermine généralement l'âge à un an de plus que les participants de la France. Cependant, l'uniformité dans la détermination de l'âge était relativement importante au Canada et en France.

INTRODUCTION

The determination of age by interpreting growth patterns on otoliths is a critical component of many biological studies and fish stock assessments. Accurate determination of fish age permits the discernment of population dynamics of multiple year-classes, which typically leads to an improved quality of management advice.

At the Northwest Atlantic Fisheries Centre, more than one hundred thousand cod otoliths have been aged. Detailed description of age determination procedures (Wells, 1977) and investigations of consistency in age determination (e.g., Batten and Wells, 1979; Hicks et al., 1998, Ferreira et al., 2006) have been reported in previous studies.

In this study we primarily investigate the consistency of age interpretations of cod in the NAFO subDivision 3Ps and also NAFO Divisions 3Pn4RS cod stocks (see Figure 1). As 3Ps cod is harvested by both France (St. Pierre et Miquelon) and Canada, this study reports on the results of age interpretations following an otolith exchange between France and Canada conducted during 2009. For reference, an annotated photograph of a cod otolith and a typical cross-section are shown in Figure 2.

Known-age fish (e.g. from tagging or captive-stock studies) are the exception when studying otoliths to determine age. When the true age is unknown, a key component of any aging program is to ensure consistency in age interpretations amongst age readers. Consistency is maintained through training of new agers and frequent exchanges between readers.

Commercial catches of 3Ps cod are sampled independently by both Canada and France. Age reading staff within DFO NL Region and IFREMER (St. Pierre et Miquelon) have many years experience in age determinations. The consistency of age interpretations between nations is informally monitored as the total international catch-at-age is compiled. The impetus for this study was some indication of potential differences in age interpretation between staff in Canada and in France. Sampling of commercial catch taken in the same area with common gear type and at approximately the same time yielded very different age distributions (Figure 3). Although it is not uncommon to have differences in age composition in similarly obtained catch, the degree of differences in this instance served as motivation to have a formal comparison of age interpretations between France and Canada. A secondary reason to initiate comparison between Canada and France was the recent retirement of a French technician in St.Pierre et Miquelon. Furthermore, the creation of IFREMER's National Sclerochronology Centre was finalized in 2007. Consequently, aging of French samples has been transferred to the National Sclerochronology Centre of the IFREMER institute at Boulogne sur Mer, France.

The aging differences noted above were reported during the 3Ps cod assessment at the winter 2009 DFO zonal cod assessment meeting and a research recommendation from this meeting was to initiate an otolith exchange (DFO, 2009; see Appendix IV). DFO Quebec region requested that the individual cod ager in Mont-Joli also participate, as this ager was trained by DFO NL staff.

METHODS

Two collections of 100 otoliths (N=200 in total) were evaluated in this study. Half of the otoliths were collected in 3Ps, the remainder in 3Pn4RS. The 3Ps cod otoliths were taken from commercial gillnet sampling conducted over January to April 2009, in unit area 3Psb. Corresponding fish length ranged from 36-96cm; with the majority of sampled fish within 50 –

70 cm (Fig. 4). The 3Pn4RS otoliths were from either sentinel or research surveys, and represented a variety of gears (gillnet, longline and otter-trawl) and a wide range of areas across 3Pn4RS. Fish size ranged from 19 – 93cm with a modal length of approximately 55 cm.

Six individuals aged the 200 otoliths considered in this study. Three of the readers were from DFO NL region (see 'HH', 'CH' and 'GC' in table and figure labels), with one reader from DFO Que region ('PS' labels) and two readers from IFREMER Boulogne sur Mer ('JF' and 'JLD' labels), France. Aging methodologies differ amongst these institutes. Detailed description of the protocols employed is given below.

At the Northwest Atlantic Fisheries Centre (NAFC) in St.John's, the otolith is scored and broken across the *sulcus acusticus* at the collum (refer to Figure 2). The otolith is viewed under transmitted light using Leica™ stereoscopic microscope at 12.5x magnification using either alcohol or Kodak™ Photo-Flo 200 solution, to change the refractive index of the otolith surface (i.e. reduce glare and increase visibility). The surface of the cut/broken otolith is shaded using a scalpel blade to ensure no reflected light from the transmitted light source obscures the view of the annuli. Occasionally the cut surface of the otolith is lightly sanded to provide a smooth viewing surface. Either the hyaline or opaque zones (under transmitted light) are used to determine age, according to reader preference.

The technique employed for preparation and viewing of the otoliths at DFO's Quebec lab is similar to that of NAFC, as the ageing knowledge was transferred from the St. John's lab to Mont-Joli in the mid-1980s. Otoliths are sectioned at the otolith nucleus using an Isomet™ low speed saw and viewed at 15x with a Leica™ stereoscopic microscope using fiber optic transmitted light. Alcohol is applied to the otolith surface. The opaque zone (under transmitted light) is considered an annulus.

The approach of the IFREMER institute in Boulogne sur Mer is to embed otoliths in polyester resin and then section these transversely at the otolith nucleus with a high speed saw (Brillant 250TM, ATM society). For each otolith, 2 to 3 sections are created with an average thickness of 0.3 mm. Age estimation is carried out with TNPC software, a program which has been developed within IFREMER. TNPC computer-assisted age and growth estimation is used routinely for acquiring and interpreting the growth structures and their storage (Mahé et al., 2009).

Multiple graphical and statistical comparisons were undertaken to evaluate consistency in age interpretations and to test for potential biases. We present the linear correlations of the age interpretations for all age readers. The percent agreement or the fraction of cases when two readers assign the same age to a common otolith was computed for each pair of readers. In addition, the mean length at age was computed from the age assigned by individual readers to determine if age determinations differ in relation to fish size. Comparisons of age interpretations to an arbitrarily-chosen 'reference reader', a common practice when investigating whether or not aging biases are present, were also conducted. Finally, we conducted a Wilcoxon pairwise nonparametric test to determine if the differences measured between readers were statistically significant (at an α=0.05 level).

RESULTS

We consider the 3Pn4RS and 3Ps otoliths independently to determine if there are any differences by stock unit, particularly as the main interest is in the consistency of aging of

commercial catches of 3Ps cod. The age determinations by the six participants for cod otoliths from 3Pn4RS and 3Ps are provided in Tables 1 & 2, respectively.

Examination of scatterplots of the ages assigned to a common otolith indicates high general agreement amongst most readers (Figures 5a and 5b). Linear correlation coefficients are near one with a high certainty of being significantly different than zero, indicating that there are no gross inconsistencies between interpretations of the six participants. This conclusion holds for both the 3Pn4RS and 3Ps samples.

Percent agreement amongst readers (Table 3) reveal similar patterns for both samples. The agreement between readers within the same institute is relatively good, and the degree of consistency between interpretations by Canadian readers (HH, CH, GC, PS) and French readers (JF, JLD) is poor.

Further indication of differences between readers is found by examining mean fish length and assigned age for each reader (Figure 6). In general, the mean lengths at age computed using the assigned age is larger for the IFREMER readers in comparison to DFO readers.

Examining the mean age as compared to a reference reader (Figures 7a and 7b) reveals some structure in the differences noted above. It is apparent that there is generally a consistent one year difference between the Canadian and French age interpretations, with the French participants assigning an age one less than their Canadian colleagues.

To quantify the significance of these differences, we computed the p-values of the Wilcoxon pairwise non-parametric test. Tabulated values indicate the significance of rejecting $H0:|X-Y|>1$, where X and Y represent the ages assigned to each otolith by a pair of readers. This formulation of the hypothesis explores the presence of a bias in age interpretation of one or more ages. For both samples, it is clear that a bias exists between the Canadian and French agers. Further, there is no bias internally amongst the four Canadian readers or the two French readers.

DISCUSSION

The 3Ps cod stock is shared jointly by Canada and France. This study was initiated to compare age interpretations made by Canadian and French age readers, for two primary reasons: 1) recent differences in age composition of commercial catches were noted when preparing the 2008 catch-at-age; 2) production of age readings of 3Ps cod by France has been transferred to the IFREMER institute in Boulogne sur Mer, France. It is important to note that any potential differences in recent years have only limited impact on the total catch-at-age, and have no impact on the aging of Canadian research vessel surveys, which are aged by Canadian staff only.

The results herein indicate an apparent one-year bias in the ages assigned by Canada and by France. This may be resulting from differences in interpretation of the initial growth ring and/or edge growth. Consequently, it is assumed that this discrepancy can be overcome by an exchange of annotated digital images or by holding a workshop.

There is also variation within the four Canadian and two French readers (as evidenced from the percent agreement values), however, differences are less than one year in most cases and there doesn't appear to be any clear bias.

The differences reported herein were common to both the 3Ps and the 3Pn4RS otoliths, indicating no stock-specific issues requiring further investigation.

We emphasize that the true age of the fish included in this study are unknown. The goal was to assess overall consistency and to detect whether any biases exist. Having reported a one year bias in the ages assigned by Canadian and French staff, we do not imply that either result is "more correct". Future collaboration between Canada and France will focus on improving consistency and reducing biases in age interpretation of Atlantic cod (*Gadus morhua*).

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Table 1. Age determinations of otoliths sampled from 3Pn4RS cod as determined by the six readers in this study.

Otolith Number	Fish Length (cm)	Assigned Age (years)					
		PS	HH	GC	CH	JF	JLD
1	37	4	4	4	4	3	3
2	39	4	5	4	5	3	3
3	49	5	5	5	5	3	3
4	53	6	7	7	7	4	4
5	53	6	7	7	7	5	4
6	55	8	9	9	10	7	7
7	37	4	5	5	4	3	3
8	40	4	5	5	5	3	3
9	41	5	5	5	5	4	4
10	46	5	6	7	5	4	4
11	48	6	6	6	7	5	5
12	46	4	6	6	6	3	4
13	52	6	6	6	7	4	5
14	45	6	6	6	6	6	6
15	46	5	6	6	6	4	4
16	54	7	7	8	8	6	7
17	55	6	6	6	6	5	5
18	59	6	6	6	6	5	5
19	62	8	9	8	9	6	7
20	63	10	10	9	10	8	9
21	64	10	9	10	10	7	9
22	66	9	10	9	10	8	9
23	68	9	10	9	10	9	9
24	70	9	9	10	9	7	8
25	71	7	8	6	8	7	8
26	72	10	10	10	10	9	9
27	73	9	9	10	10	8	8
28	78	11	11	11	11	9	9
29	84	10	10	10	10	8	8
30	93	11	11	12	11	10	10
31	48	5	6	6	5	4	4
32	51	6	6	6	6	5	5
33	56	8	8	8	9	6	7
34	57	11	12	11	12	10	10
35	59	9	10	9	10	8	8
36	62	9	9	9	9	8	8
37	64	9	9	9	10	8	8
38	66	13	13	14	12	11	11
39	68	7	8	7	8	6	6
40	70	9	10	9	10	7	8
41	28	3	2	3	3	2	2
42	31	3	3	3	3	2	2
43	32	3	3	3	3	2	2
44	36	4	3	3	4	3	3
45	36	3	3	3	3	3	2
46	37	4	4	4	4	3	3
47	38	4	4	4	4	3	3
48	40	4	4	4	4	3	3
49	40	4	4	4	4	4	3
50	43	5	5	4	4	4	4
51	44	4	4	4	4	4	4
52	44	5	5	5	4	4	4
53	49	5	4	5	5	4	4
54	52	7	7	7	7	6	6
55	61	9	9	9	8	7	8
56	19	2	2	2	3	1	1
57	20	2	2	2	3	1	1
58	21	2	2	2	3	1	1
59	23	2	2	2	3	1	1
60	27	2	2	3	3	2	2
61	32	3	3	3	4	2	2
62	38	4	4	4	4	3	2
63	52	7	7	7	7	4	5
64	55	6	7	7	8	5	5
65	57	6	6	6	6	5	5
66	57	7	7	7	8	5	6
67	58	8	8	8	8	6	5
68	59	8	8	8	9	7	7
69	60	6	6	6	7	6	6
70	68	9	9	9	10	7	8
71	70	9	9	9	10	8	9
72	74	9	9	9	10	8	8
73	91	13	13	13	13	11	11
74	27	2	2	2	3	2	1
75	30	2	2	3	3	2	2
76	34	3	3	3	3	3	2
77	37	4	4	4	4	3	3
78	42	5	4	4	5	4	4
79	52	5	6	5	6	4	4
80	56	5	5	6	6	4	4
81	52	8	9	9	9	6	7
82	53	5	5	6	6	5	4
83	55	6	7	6	7	5	5
84	65	8	8	8	9	6	7
85	66	9	9	10	10	7	8
86	67	8	7	8	8	6	6
87	69	10	10	10	10	8	10
88	71	14	14	14	15	12	13
89	72	9	9	10	10	8	9
90	85	11	11	11	11	9	12
91	43	4	5	4	6	5	5
92	45	4	4	4	4	4	4
93	52	6	6	6	6	5	5
94	54	8	8	9	8	7	8
95	57	8	8	8	8	6	6
96	58	9	9	9	9	6	7
97	59	9	9	9	10	8	9
98	62	6	6	7	7	5	6
99	63	9	9	10	9	8	9
100	81	10	11	10	12	8	10

Table 2. Age determinations of otoliths sampled from 3Ps cod as determined by the six readers in this study.

Otolith Number	Fish Length (cm)	Assigned Age (years)					
		PS	HH	GC	CH	JF	JLD
1	46	5	6	5	5	4	4
2	47	5	6	5	5	5	4
3	49	6	7	7	7	6	5
4	52	6	6	7	7	7	6
5	53	8	9	9	9	8	8
6	56	9	9	9	9	7	7
7	57	8	9	9	9	10	10
8	59	8	9	9	9	9	10
9	60	9	8	9	9	8	8
10	61	8	8	8	8	9	9
11	64	9	11	11	11	11	11
12	65	12	12	13	12	13	13
13	66	7	7	7	8	8	6
14	67	9	9	9	10	8	9
15	68	11	12	11	12	9	10
16	69	9	11	11	11	9	9
17	71	13	15	15	15	13	13
18	72	8	8	8	8	8	8
19	79	14	15	15	15	13	13
20	91	12	13	13	13	12	12
21	96	12	13	13	13	12	12
22	44	6	6	5	6	5	5
23	44	5	5	5	5	4	4
24	45	6	7	6	7	5	4
25	49	5	6	5	6	5	4
26	49	6	6	6	6	5	5
27	50	6	6	6	6	4	4
28	50	8	8	8	8	7	7
29	51	7	8	8	9	6	5
30	71	7	7	6	7	5	5
31	72	8	8	8	8	7	6
32	72	9	9	10	9	7	6
33	74	12	12	12	13	11	11
34	79	10	11	10	11	9	9
35	81	10	10	10	10	9	7
36	82	11	12	12	12	10	10
37	44	5	5	5	5	4	4
38	53	9	9	9	10	8	7
39	71	8	7	7	9	9	9
40	73	11	11	11	11	10	9
41	91	13	14	14	14	13	13
42	50	7	6	7	7	6	5
43	52	8	9	8	9	7	7
44	53	7	8	9	9	8	7
45	54	8	7	8	10	8	8
46	56	10	11	11	11	8	8
47	58	9	10	12	11	10	8
48	60	7	8	7	8	9	9
49	61	8	8	9	9	7	6
50	62	7	8	8	8	7	6
51	63	8	9	8	9	7	7
52	65	12	12	12	12	11	11
53	66	7	6	7	7	6	6
54	67	7	7	7	7	7	6
55	68	13	14	14	14	13	12
56	70	12	14	14	14	12	11
57	72	9	9	9	9	8	9
58	81	10	11	12	12	6	4
59	40	4	5	5	5	4	4
60	40	4	5	5	5	4	3
61	42	5	6	5	5	4	4
62	43	5	5	5	5	4	4
63	51	5	6	6	6	5	4
64	51	9	11	12	10	8	8
65	53	7	7	7	7	6	6
66	54	7	7	7	7	6	6
67	55	8	8	10	9	7	8
68	56	9	9	10	9	8	8
69	57	10	10	11	11	9	9
70	57	9	9	9	9	8	7
71	60	9	9	9	9	8	8
72	63	8	8	8	8	7	7
73	65	6	7	7	7	6	5
74	66	11	11	14	14	10	10
75	67	7	7	7	7	6	6
76	67	12	13	13	13	12	11
77	68	9	9	8	10	11	10
78	69	9	9	9	9	8	8
79	69	9	9	10	10	8	8
80	70	9	9	9	10	8	8
81	81	8	10	10	11	9	9
82	39	4	4	5	6	5	3
83	43	5	6	6	6	6	5
84	44	5	5	5	5	4	4
85	46	5	5	5	5	4	4
86	46	5	5	6	7	5	5
87	47	7	7	7	7	6	6
88	48	7	7	7	7	6	6
89	49	5	6	6	6	4	4
90	51	6	6	8	7	5	4
91	52	9	9	9	9	8	8
92	53	8	9	8	8	8	8
93	55	6	6	6	7	6	6
94	57	7	7	7	8	7	7
95	58	9	10	10	10	11	10
96	59	7	8	7	9	6	6
97	61	8	8	8	9	7	7
98	62	11	11	11	12	10	10
99	66	8	8	8	9	7	7
100	68	10	11	10	11	10	10

Table 3. Percent agreement between readers. Values in dashed boxes indicate readers at the same institute.

3Pn4RS Otoliths

	HH	CH	GC	PS	JF	JLD
HH	100	55	66	70	16	21
CH	55	100	53	47	8	10
GC	66	53	100	70	12	14
PS	70	47	70	100	13	18
JF	16	8	12	13	100	63
JLD	21	10	14	18	63	100

3Ps Otoliths

	HH	CH	GC	PS	JF	JLD
HH	100	64	64	52	13	12
CH	64	100	65	39	10	7
GC	64	65	100	54	16	12
PS	52	39	54	100	25	20
JF	13	10	16	25	100	64
JLD	12	7	12	20	64	100

Table 4. Paired Wilcoxon (nonparametric) test statistic p-values for testing if age interpretations amongst readers is statistically significant. Table entries are p-values of testing H0: $|x-y| \geq 1$ for the pair of readers identified by the row and column labels.

3Pn4RS Cod

	p-value				
	CH	**GC**	**PS**	**JF**	**JLD**
HH	1	1	1	0	0
CH		1	0.999	0	0
GC			1	0	0
PS				0	0
JF					1

3Ps Cod

	p-value				
	CH	**GC**	**PS**	**JF**	**JLD**
HH	1	1	1	0	0
CH		1	0.622	0	0
GC			0.998	0	0
PS				0.013	0
JF					1

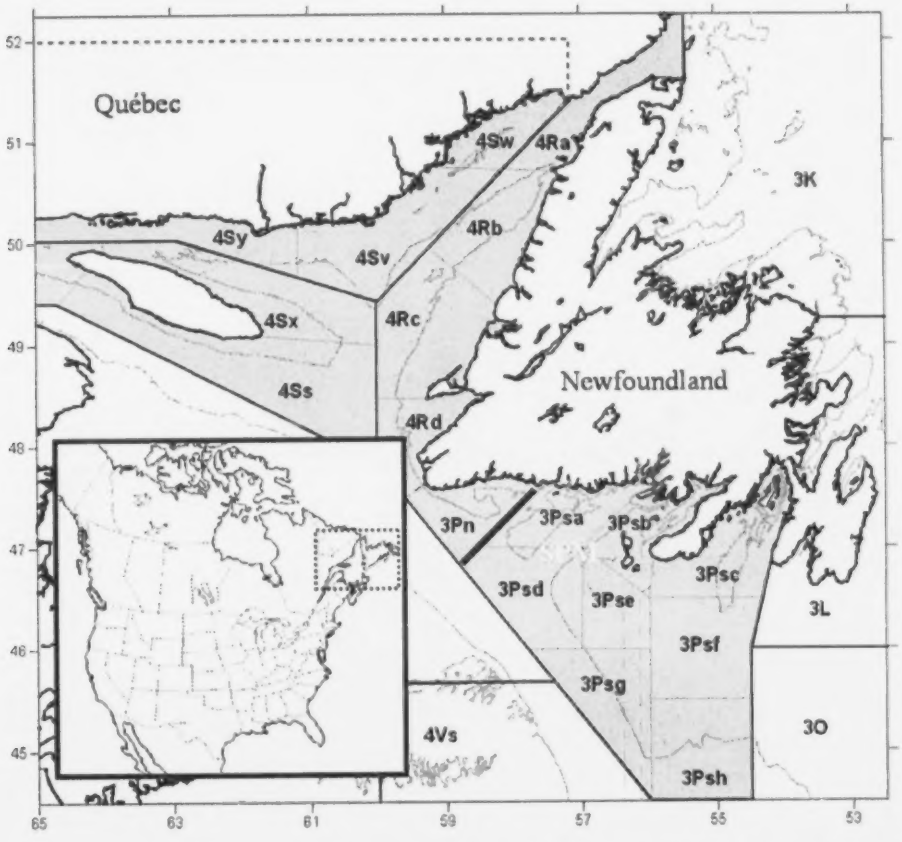


Figure 1. Map of study area. Otoliths were collected from the NAFO subdivision 3Ps cod stock (adjacent to southern Newfoundland and the French islands of St.Pierre et Miquelon), and from the NAFO Division 3Pn4RS cod stock in the Gulf of St.Lawrence.

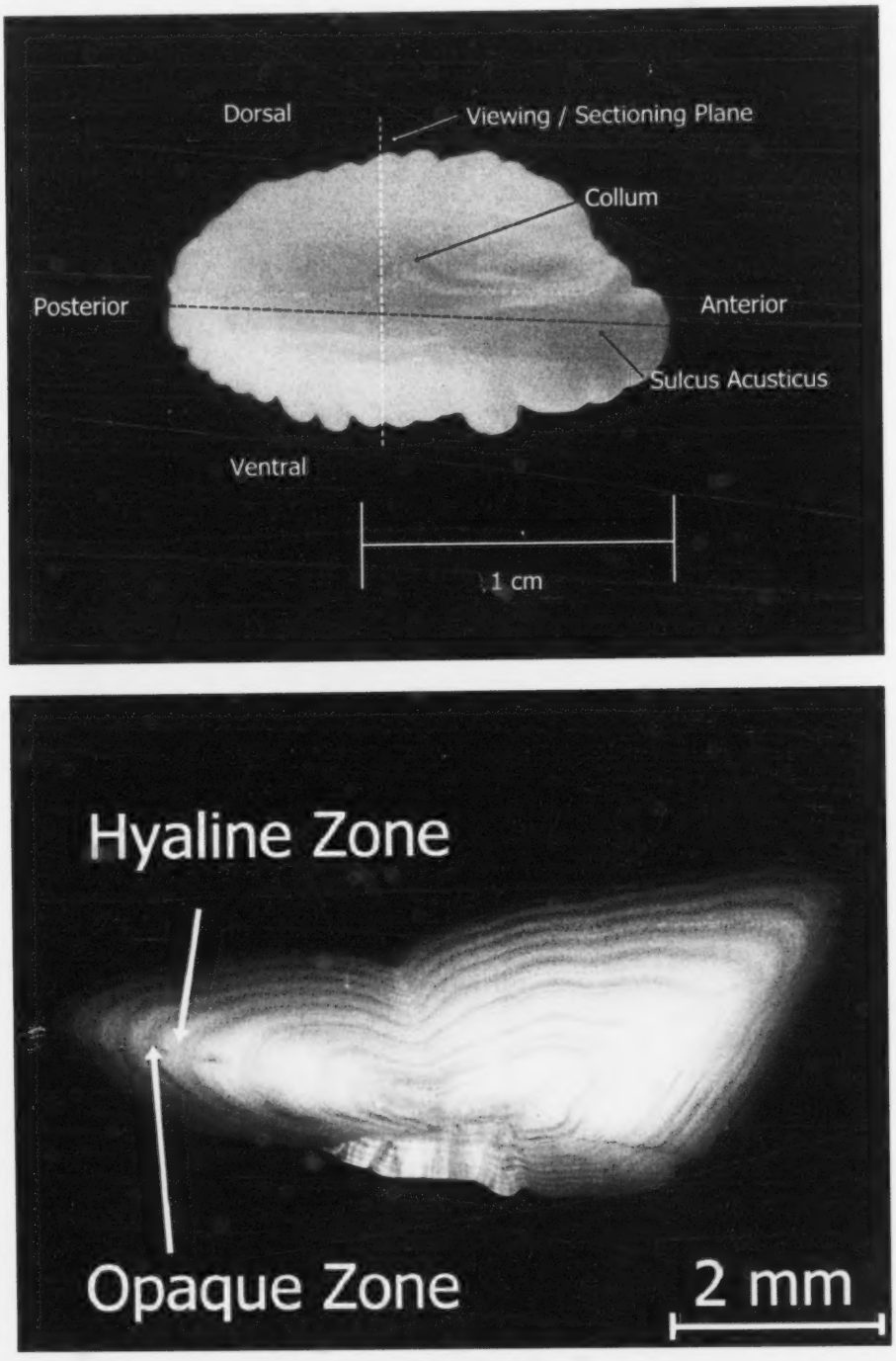


Figure 2. Upper panel: Atlantic cod otolith (convex side). Lower panel: Cross-section of Atlantic cod otolith.

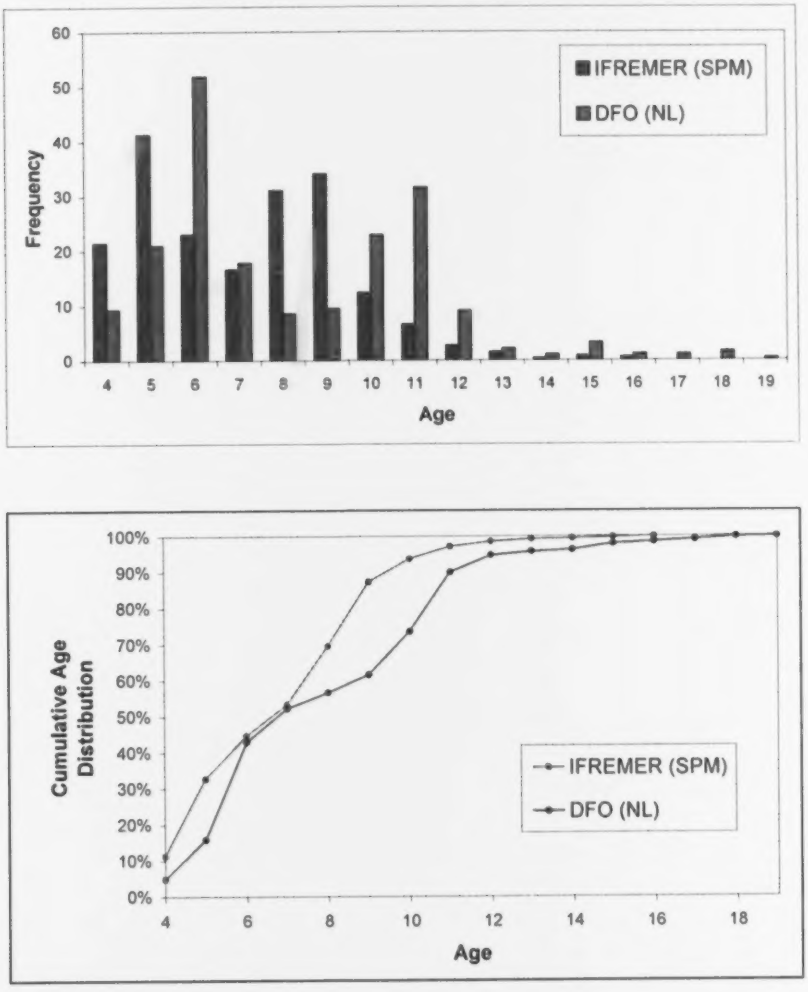
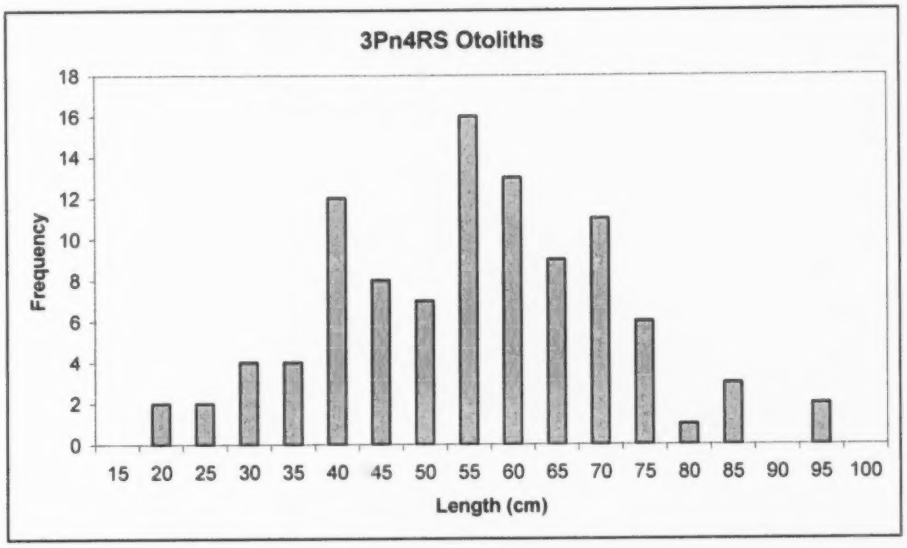


Figure 3. Comparison of age distribution assigned to 3Ps cod commercial catches. French and Canadian age interpretations were applied to a common length sample to produce the catch at age (upper panel) and cumulative age distribution (lower panel). Samples of catch were obtained from ottertrawl fishing in unit area 3Psh during the first quarter of 2008.



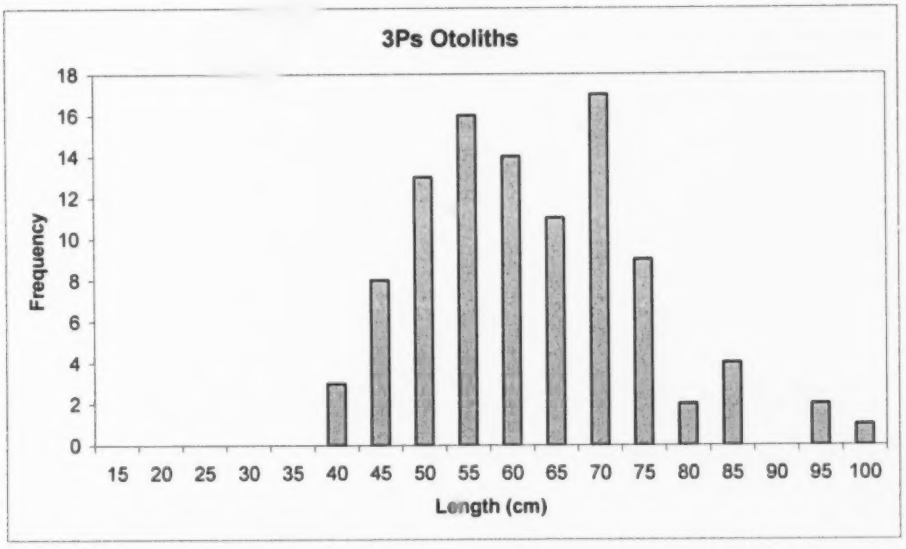


Figure 4. Fish length (cm) of cod from which otoliths used in this study were collected.

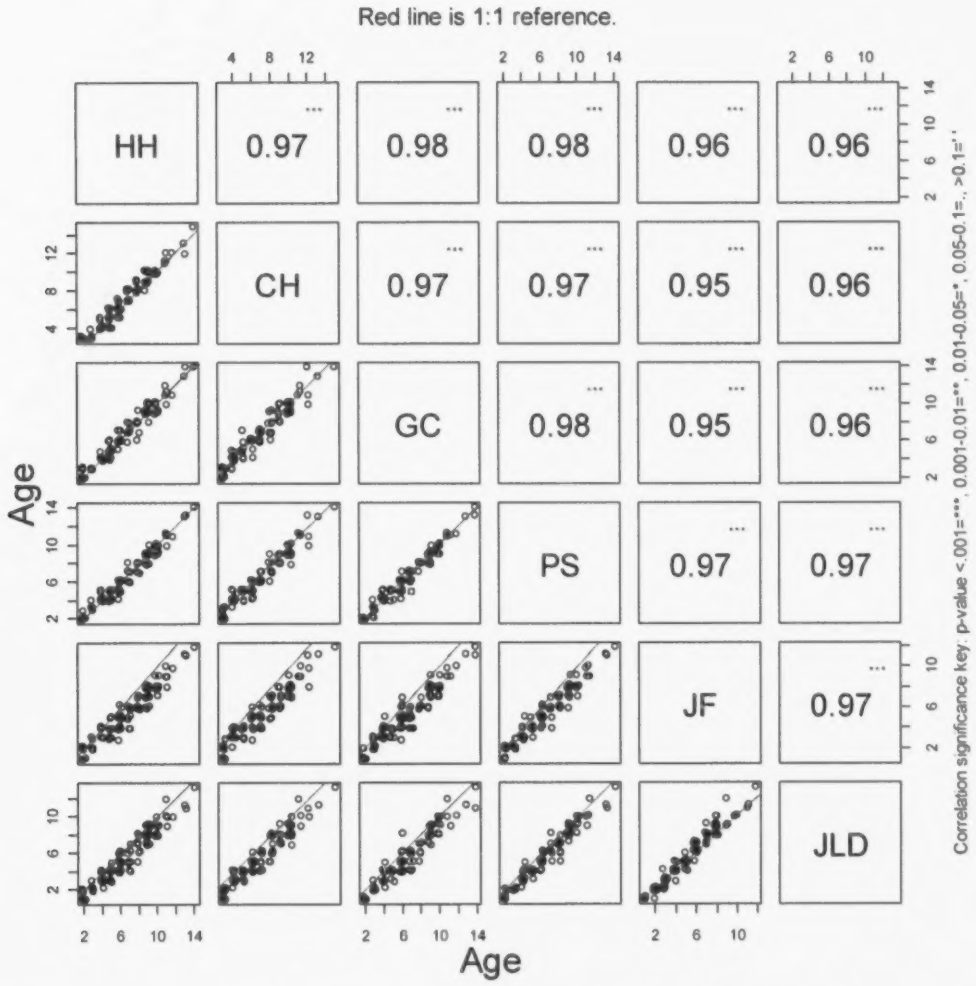


Figure 5a. Age interpretations of 3Pn4RS cod. Lower diagonal panels: Scatterplot of assigned age for a common otolith for each pair of readers. Upper diagonal panels: Linear correlation coefficients between ages assigned by each pair of readers. Significance of testing rho=0 identified by key on right hand side.

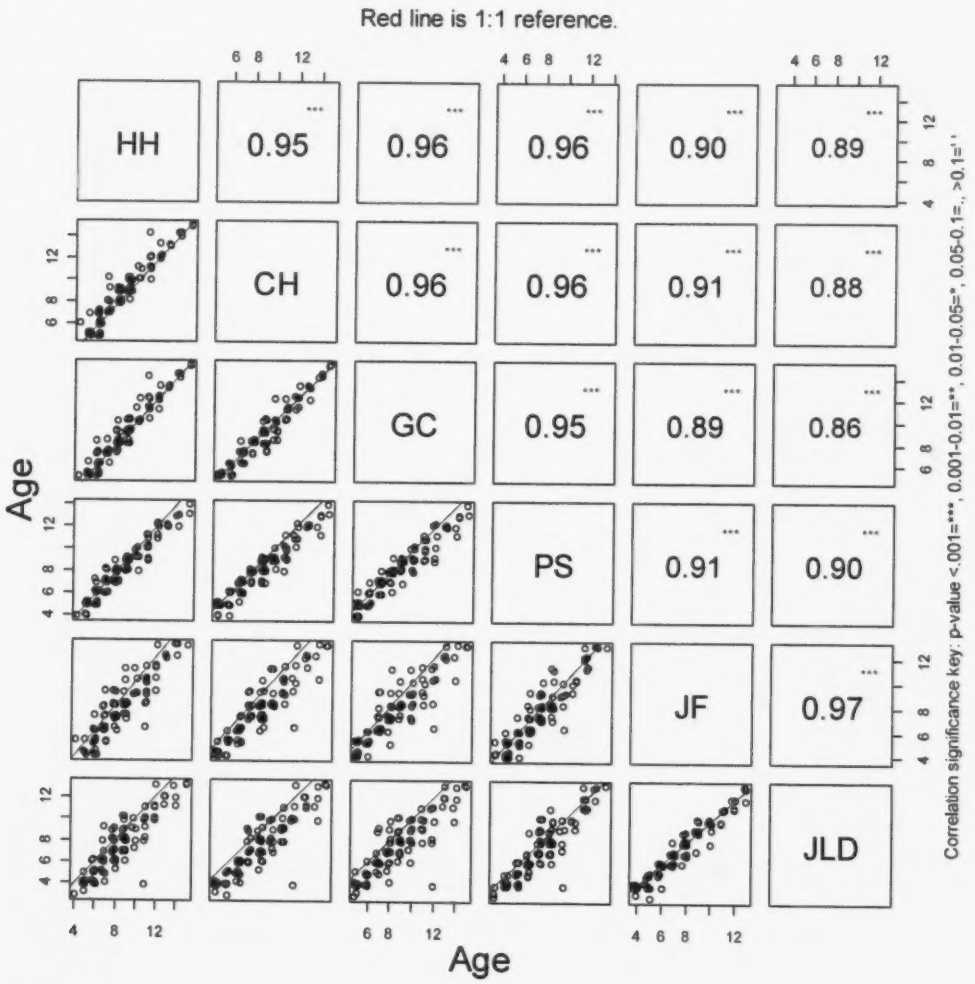


Figure 5b. Age interpretations of 3Ps cod. Lower diagonal panels: Scatterplot of assigned age for a common otolith for each pair of readers. Upper diagonal panels: Linear correlation coefficients between ages assigned by each pair of readers. Significance of testing rho=0 identified by key on right hand side.

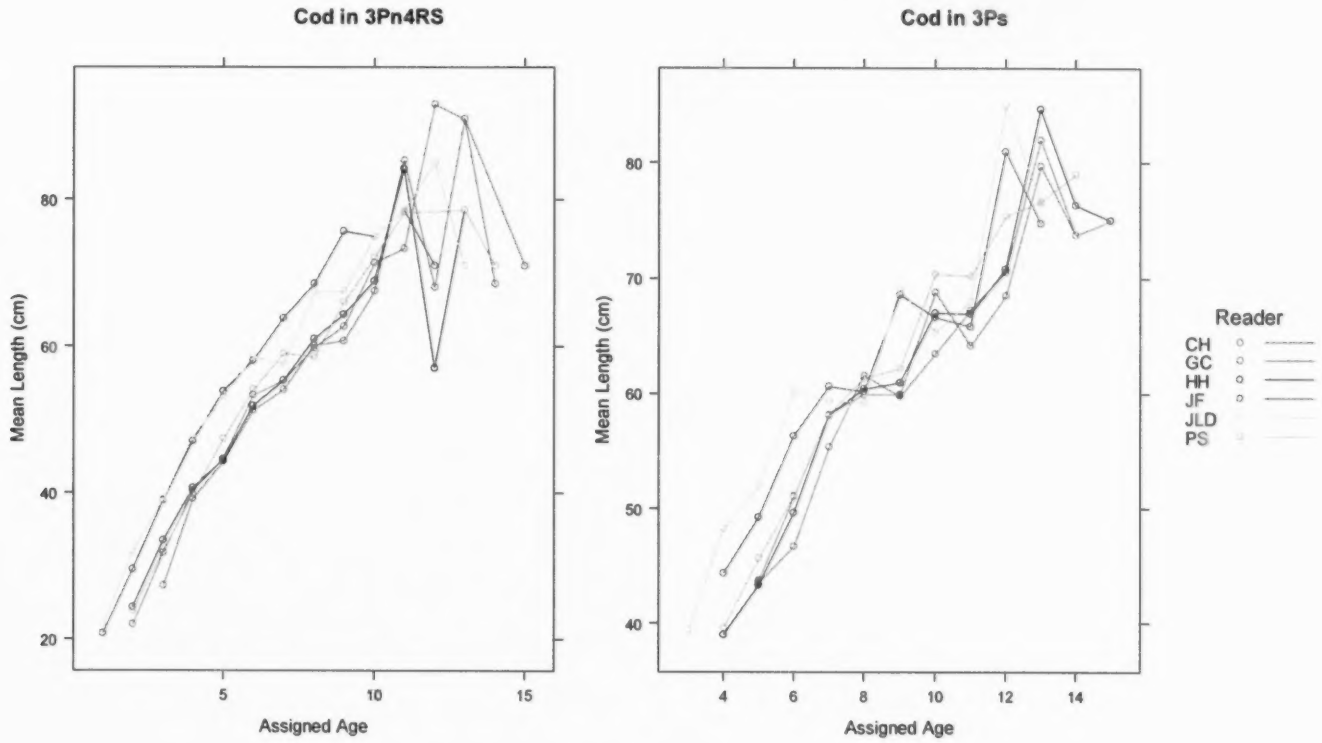


Figure 6. Mean length (cm) at age computed from the age assigned by individual reader.

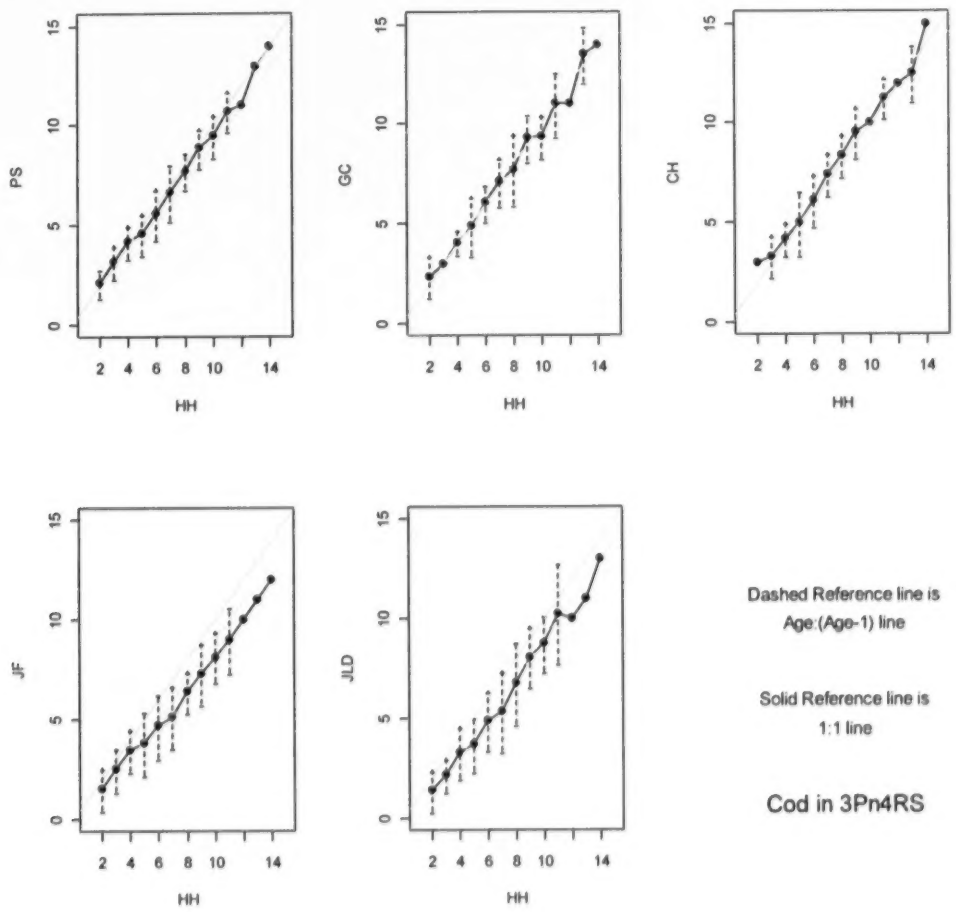


Figure 7a. Mean age (filled circle) +/- 2 standard deviations of assigned ages relative to the reference reader age. Two reference lines are plotted; a 1-1 reference (soild grey line) and an 'age : (age-1)' reference (dashed grey line).

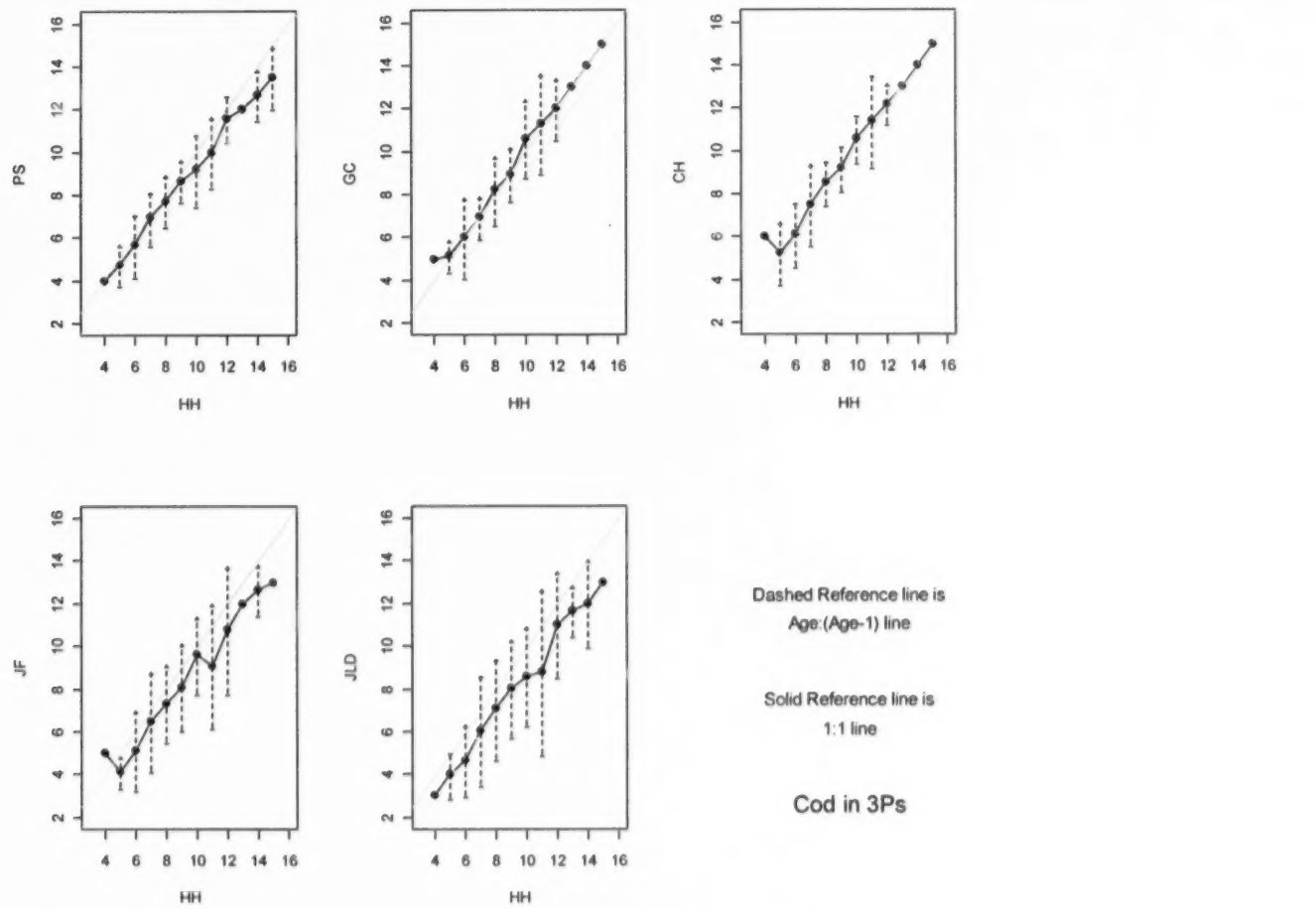


Figure 7b. Mean age (filled circle) +/- 2 standard deviations of assigned ages relative to the reference reader age. Two reference lines are plotted; a 1-1 reference (soild grey line) and an 'age : (age-1)' reference (dashed grey line).